ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

OFFICE OF PESTICIDE
PROGRAMS
Health Effects Division

September 1, 1999

MEMORANDUM

Subject: **Parathion (057501).** The Outcome of the HED Metabolism Assessment Review Committee Meeting Held on August 24, 1999 to Re-evaluate the Relative Toxicity of 4-Acetamidoparaoxon to Parathion. DP Barcode: D258694.

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And

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Through: Richard Loranger, Chair
Metabolism Assessment Review Committee
Health Effects Division [7509C]

And

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To: George Kramer, Executive Secretary
Metabolism Assessment Review Committee
Health Effects Division [7509C]

Background

On March 11, 1998, the HED Metabolism Assessment Review Committee (memo by B. Cropp-Kohlligian dated 5/21/98) met to discuss the available parathion plant and animal metabolism data (animal magnitude of the residue data were not available) and concluded the following. Parathion residues of concern in plant commodities include parathion, its metabolite paraoxon [*O,O*-diethyl-*O*-*p*-nitrophenyl phosphate], and *p*-nitrophenol and that parathion residues of concern in animal commodities include parathion, paraoxon, *p*-nitrophenol, and 4-acetamido-paraoxon. The tolerance expression for plant and animal commodities should be based on parathion only. Parathion residues of concern to be included in the risk assessment for plant commodities with regard to cholinesterase inhibition should include parathion and paraoxon. Parathion residues of concern to be included in the risk assessment for animal commodities with regard to cholinesterase inhibition should include parathion, paraoxon, and 4-acetamidoparaoxon. Residues of *p*-nitrophenol resulting from the use of parathion do not have to be included in the tolerance expression or considered in the aggregate risk assessment for parathion with regard to cholinesterase inhibition, but may be considered in conjunction with a future cumulative risk assessment for *p*-nitrophenol based on its own toxicological endpoints (rather than cholinesterase).

At that same meeting, the HED Metabolism Assessment Review Committee conjectured that although paraoxon was a powerful cholinesterase inhibitor, 4-acetamidoparaoxon might be expected to be a less powerful inhibitor of cholinesterase, based on the electronic properties of the acetamido group. It was felt, however, that in the absence of experimental data it was not possible to verify this conjecture and determine to what extent cholinesterase inhibitory activity would be decreased in the case of the acetamido metabolite. The Committee noted that the high acute oral toxicity of paraoxon [oral LD_{50} = 3.5 mg/kg (male and female rats), DuBois et al.1949] is attributed to the high cholinesterase inhibitory activity of the compound and that a decreased cholinesterase inhibitory activity on the part of 4-acetamidoparaoxon would be reflected in its having a lower acute oral LD_{50} in rats.

Thus, the HED Metabolism Assessment Review Committee (memo by B. Cropp-Kohlligian dated 5/21/98) further concluded, as had an earlier HED Metabolism Committee (memo by S. Hummel dated 2/10/95), that if the registrant could demonstrate that 4-acetamidoparaoxon is much less acutely toxic than parathion by providing acute oral toxicity data demonstrating that the LD_{50} for 4-acetamidoparaoxon is more than 200 mg/kg, then 4-acetamidoparaoxon residues incurred in animal commodities would not need to be included in the dietary risk assessment for parathion. Subsequently, the registrant (Cheminova) submitted acute oral toxicity data (MRID 44888901) to address the Agency's concerns regarding the toxicity of 4-acetamidoparaoxon as well as animal magnitude of the residue data (MRIDs 44527301 and 44527302) to estimate the residue levels of 4 acetamidoparaoxon in animal commodities.

Question to the Committee

Based on the acute oral toxicity data for 4-acetamidoparaoxon (MRID 44888901) and parathion, are residues of 4-acetamidoparaoxon incurred in animal commodities resulting from exposure of livestock to residues of parathion in/on feed items of sufficient toxicological concern with regards to cholinesterase inhibition that residues of 4-acetamidoparaoxon must be included in the dietary risk assessment for parathion?

Individuals in Attendance

Metabolism Committee:	Richard Loranger (Chair), Alberto Protzel, and William Wassell (acting Secretary).
Scientists:	Bonnie Cropp-Kohlligian, Richard Griffin, and Nicole Paquette.

Materials Reviewed

The Committee reviewed the information summarized in the attached briefing paper, which focused on oral acute toxicity data demonstrating the LD_{50} for parathion and 4-acetamidoparaoxon and animal magnitude of the residue data.

Conclusion(s) of the Committee

Based on the weight of evidence demonstrating that 4-acetamidoparaoxon was a considerably weaker cholinesterase inhibitor compared to parathion, as evidenced by the absence of lethality in the submitted acute toxicity study, the HED Metabolism Review Committee concluded that residues of 4-acetamidoparaoxon incurred in animal commodities resulting from exposure of livestock to residues of parathion in/on feed items does not have to be included in the dietary risk assessment for parathion with regard to cholinesterase inhibition. The Committee noted that although 4-acetamidoparaoxon comprises most of the total residue in milk, the dietary risk is not significant when the lower toxicity and estimated residue level (1 ppb) of that metabolite are taken into account.

Attachment 1: Parathion (057501). Issue to be Presented to the HED Metabolism Assessment Review Committee 8/24/99: Re-evaluation of the Relative Acute Oral Toxicity of 4-Acetamidoparaoxon to Parathion. MRID 44888901

Note: Since the subject 4-acetamidoparaoxon acute oral toxicity data (MRID 44888901) were not submitted to satisfy guideline requirements (OPPTS GLN 870.1100), but specifically to address HED Metabolism Review Assessment Committee concerns, the attached "issues paper" provides sufficient details of the 4-acetamidoparaoxon acute toxicity data (MRID 44888901) to be considered a full review of these data. A detailed review of the submitted animal magnitude of the residue data (MRIDs 44527301 and 44527302) will follow under separate cover.

cc (w/attachment): BLCKohlligian (RRB2/HED), RGriffin (RRB2/HED), NPaquette (RRB2/HED, RLoranger (MARC/HED), DDeziel (SRRD), Parathion Reg. Std. File, Parathion SF, RF.
RDI: HED MARC members:
7509C:RRB2:BLCKohlligian:CM#2:Rm 712N:703-305-7462:8/30/99.

ATTACHMENT 1

ENVIRONMENTAL PROTECTION AGENCY

Washington, D.C. 20460

OFFICE OF PESTICIDE
PROGRAMS
Health Effects Division

August 24, 1999

MEMORANDUM

Subject: **Parathion (057501).** Issue to be Presented to the HED Metabolism Assessment Review Committee 8/24/99: Re-evaluation of the Relative Acute Oral Toxicity of 4-Acetamidoparaoxon to Parathion. MRID 44888901.

From: Nicole Paquette, Ph.D.
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To: George Kramer, Executive Secretary
Metabolism Assessment Review Committee
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QUESTION TO THE COMMITTEE: Based on the acute oral toxicity data for 4-acetamido-paraoxon (MRID 44888901) and parathion in conjunction with other relevant evidence, are residues of 4-acetamidoparaoxon incurred in animal commodities resulting from exposure of livestock to residues of parathion in/on feed items of sufficient toxicological concern with regards to cholinesterase inhibition that residues of 4-acetamidoparaoxon must be included in the dietary risk assessment for parathion? What additional toxicology data, if any, are required to further address this question?

Background

On March 11, 1998, the HED Metabolism Assessment Review Committee (MARC) met, in part, to discuss and determine which parathion residues found in animal commodities are of toxicological concern and need to be included in the dietary risk assessment for parathion based on cholinesterase inhibition (memo by B.Cropp-Kohlligian to G.Kramer dated 5/21/98) . The Committee concluded that, based on available animal metabolism data, parathion residues of concern in animals commodities which need to be included in the dietary risk assessment for parathion with regard to cholinesterase inhibition include parathion, paraoxon, and 4-acetamido-paraoxon.

In its deliberations of March 11, 1998, the HED MARC conjectured that although paraoxon was a powerful cholinesterase inhibitor, 4-acetamidoparaoxon might be expected to be a less powerful inhibitor of cholinesterase, based on the electronic properties of the acetamido group. It was felt, however, that in the absence of experimental data it was not possible to verify this conjecture and determine how much cholinesterase inhibitory activity would be decreased in the case of the acetamido metabolite. The Committee noted that the high acute oral toxicity of paraoxon [oral LD_{50} = 3.5 mg/kg (♂ &♀ rats), DuBois et al.1949] is attributable to the high cholinesterase-inhibitory activity of the compound and that a decreased cholinesterase inhibitory activity of 4-acetamidoparaoxon would be reflected in a decreased acute oral LD_{50} in rats.

Thus, the Committee further concluded, as had an earlier HED Metabolism Committee (memo by S. Hummel dated 2/10/95), that if the registrant could demonstrate that 4-acetamidoparaoxon (4-AAP) was much less acutely toxic than parathion by demonstrating that the acute oral LD_{50} of 4-AAP is more than 200 mg/kg, then residues of 4-AAP would not need to be included in the dietary risk assessment for parathion.

Subsequently, the registrant (Cheminova) submitted acute oral toxicity data on 4-acetamidoparaoxon in rats (MRID 44888901) to specifically address the HED Metabolism Review Committee's concerns regarding the relative acute oral toxicity of 4-AAP to parathion. [Note: The subject study was not submitted to satisfy acute oral toxicity guideline requirements (OPPTS GLN 870.1100) and has not been reviewed herein for that purpose.]

Current Considerations

Acute Oral Toxicity Data for 4-Acetamidoparaoxon (MRID 44888901) and Parathion

Executive Summary: In an acute oral toxicity study (MRID 44888901), one group of three female Sprague-Dawley was given a single gavage dose of 200 mg/kg of 4-acetamido-paraoxon (4-AAP) in distilled water in a 10 ml/kg volume. Females were used because it has been well documented that female rats are more sensitive to the acute lethal effects of the parent compound, parathion. The animals were observed for deaths and overt signs of toxicity at ½, 1, 24 hours and subsequently once daily for 14 days. Individual body weights were recorded prior to dosing and

at 7 and 14 days following treatment.

None of the three female rats given 200 mg/kg died. There were no decreased bodyweight throughout the treatment period. All three had clinical signs of cholinergic toxicity including hunched posture (3/3), lethargy (3/3) piloerection (2/3). Clinical signs occurred within ½ to 1 hour of dosing but totally disappeared by Day 2 of observation. One female also had labored breathing with a decreased respiratory rate and was ataxic (uncoordinated movements) which occurred within ½ hour of dosing which disappeared by the 24 hour observation period. There were no abnormalities noted at necropsy on Day 14.

Since none of the three female rats died given 200 mg/kg, the acute lethal dose (LD_{50}) for 4-AAP appears to be greater than 200 mg/kg bw.

This study was not submitted to satisfy guideline requirements (OPPTS 870.1100) and was considered for the specific purpose of addressing HED Metabolism Assessment Review Committee's concern regarding the acute lethality potential of 4-AAP relative to parathion.

I. Materials and Methods:

A. Materials:

1. Test Material:
   Description: yellow crystalline solid
   Lot/Batch #: 250-ABB-32
   Purity: 92.4% a.i.
   Verification of concentration/homogeneity; Cheminova Batch Analytical Certificate, NMR analysis

2. Vehicle : Distilled Water

3. Test animals:
   Strain: Female Sprague Dawley CD (Crl:CD®(SD) IGS BR)
   Age and/or weight at dosing: 8 to 12 weeks old; 207 - 211 g
   Source: Charles River (UK) Ltd,
   Acclimation period: 5 days
   Diet: Rat & Mouse Expanded diet #1 ad libitum
   Water: ad libitum
   Housing: Polypropylene Cage with woodflakes
   Environmental conditions: Temperature: 19 - 25°C
   Humidity: 30 - 70%
   Photoperiod: 12 hour light/dark

B. Study Design and Methods:

1. In life dates - start: June 2, 1999 end: June 16, 1999

2. Animal assignment and treatment -Following an overnight fast, 3 female rats were given a single dose of 200 mg/kg bw by gavage then observed at ½ 1, 2, 4 hours and subsequently daily and weighed at day 7 and 14. A necropsy was performed on survivors.

II. Results and Discussion:

A. Mortality is given in table 1.

Table 1. Individual Data

Dose (mg/kg	Rats Treated	Deaths During Day of Dosing (Hours)				Deaths During Period After Dosing (Days)								Deaths
		½	1	2	4	1	2	3	4	5	6	7	8-14	
200	3 ♀	0	0	0	0	0	0	0	0	0	0	0	0	0/3

B. Clinical observations -

All three female rats had hunched posture and were lethargic by the first hour following treatment which lasted through the second day. Two of the three rats also had piloerection the first day which disappeared 24 hours later. One female rat had labored breathing with decreased respiration rate within ½ hour of dosing and at all other time points on the first day of observation. The same female also was ataxic by the fourth hour of observation but not noted the following day. By day 2, all three animals recovered totally from all clinical signs.

Dose Level	Animal Number	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)				
		1/2	1	2	4	1	2	3	4	5-14
200 (mg/kg)	1-0	HLRd Rl	HLRd Rl	HLRd Rl	HLRdA Rl	HL	0	0	0	0
	2-0	0	H	HLP	HLP	H	0	0	0	0
	3-0	0	H	HL	HL	H	0	0	0	0

0 = no signs of systemic toxicity P = piloerection
A = ataxia Rd = decreased respiratory rate
H = hunched posture Rl = labored respiration

C. Body Weight -

There were no decreased body weight or body weight gain at any time point measured during the entire study.

Dose Level (mg/kg)	Animal Number	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
200	1-0	211	258	275	47	17
	1-1	210	249	261	39	12
	1-2	207	225	225	18	5

D. Necropsy -

There were no abnormalities noted at necropsy.

Summary Table of Acute Oral Toxicity Data for 4-AAP Relative to Ethyl Parathion

Parameter	**4-AAP**	**Parathion**
LD_{50} in females	200 mg/kg n=3	2 - 4 mg/kg n=5
Clinical Signs	hunched posture (3) lethargy (3) piloerection (3) labored breathing (1)	hunched posture (3) lethargy (4) piloerection (5) labored breathing (1) soiled fur (5) clonic convulsions (5)
# of Deaths/Day	No Deaths (0/3)	2 mg/kg = 2/5 dead the first day 4 mg/kg = 4/5 dead by first day
Onset of Cholinergic Symptoms	½ hour	½ hour
Recovery from Symptoms	All recovered by Day 2	lethargy, piloerection, soiled fur remained until Day 5

The parent compound, parathion, is extremely toxic and lethal in female rats acutely, regardless of the route of administration.

The LD_{50} for female rats = **2-4 mg/kg**; LD_{50} for male rats = ~20 mg/kg

The present information indicates that the metabolite, 4-acetamidoparaoxon, is considerably less lethal in female rats by the oral route:

The Approximate LD_{50} for female rats ~ **200 mg/kg**

The active metabolite, paraoxon, has an acute oral LD_{50} of 3.5 mg/kg for both male and female rats. Thus is expected that acute oral toxicity of 4-acetamidoparaoxon in both sexes should be comparable.

Although an acute oral toxicity study only tells us of the lethal potential of the chemical, we assume that with organophosphates, lethality occurs as a result of cholinergic overstimulation caused by inhibition of acetylcholinesterase. It is assumed that at high enough doses (LD_{50}) this prolonged cholinergic response ultimately results in paralysis of the respiratory muscles leading to death. For parathion the "assumed" cholinergic-induced lethality in female rats is approximately 2-4 mg/kg whereas for 4-AAP the "assumed" cholinergic-induced lethality is approximately 200 mg/kg. This makes the cholinesterase-inhibiting properties of 4-AAP considerably less potent compared with the parent, parathion.

Animal Magnitude of the Residue Data (MRIDs 44527301 and 44527302)

Based on the maximum theoretical dietary burdens for beef and dairy cattle (8 ppm and 10 ppm, respectively) and the available ruminant (cow) magnitude of the residue data, residues of 4-acetamidoparaoxon are estimated at 1.0 ppb in milk, 0.9 ppb in beef muscle, 1.0 ppb in beef kidney, 1.1 ppb in beef fat, and 11 ppb in beef liver. Based on the maximum theoretical dietary burden for poultry (3.8 ppm) and the available poultry (hen) magnitude of the residue data, residues of 4-acetamidoparaoxon are estimated at 0.09 ppb, 0.1 ppb in poultry muscle, 0.2 ppb in poultry fat, and 6 ppb in poultry liver. Estimates for the residues of 4-acetamidoparaoxon in beef liver and poultry liver are likely to be over estimations since residue levels of 4-acetamidoparaoxon found in liver test samples have been corrected (conservatively) for potential residue decline due to test sample storage.